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Combined RCT – aggregated N-of-1 trial protocol: GAG-therapy Efficacy Trial Solution for Bladder pain syndrome/ Interstitial Cystitis (GETSBI study).

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Title: Combined RCT – aggregated N-of-1 trial protocol: GAG-therapy Efficacy Trial Solution for Bladder pain syndrome/ Interstitial Cystitis (GETSBI study).

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Abstract

Introduction Obtaining level 1 evidence on efficacy of GAG-therapy is difficult, due to low incidence of BPS/IC and heterogeneous symptoms experienced by BPS/IC patients. Currently, because of a lack of high-grade evidence, the recommendation for applying GAG-replenishment therapy in most guidelines is 'low grade'. An Aggregated N-of-1 trial is a multi-crossover design that yields similar level 1 evidence as a traditional Randomized Controlled Trial (RCT), while requiring far less patients. The goal of this study is to investigate the efficacy of intravesical GAG therapy (IALURIL®) for bladder pain syndrome patients with Hunner lesions using a dual RCT and aggregated N-of-1 trial design to obtain Level 1 evidence.

Methods and analysis The GETSBI study is a double-blind multi-design multi-centre randomized placebo-controlled study to assess the short and long-term efficacy of Hyaluronic acid (1.6%) + Chondroitin sulfate (2%) therapy (Ialuril ® Prefill, IBSA, Goodlife) in symptomatic BPS/IC patients with Hunner lesions. The study protocol is based and powered on a standard RCT, but continues as an aggregated N-of-1 trial. The patients will be randomized in one of the three parallel arms, receiving blinded treatment for three periods (1x/week for six weeks, ratio placebo to intervention in periods of 2:1). Followed by an open prospective part for the long-term efficacy of GAG-therapy. The primary study outcome is the maximum bladder pain experienced in the last 3 days measured using the VAS pain scale (0-10).

Ethics and dissemination This study is initiated in collaboration with the Dutch government. It will deliver evidence of efficacy of GAG-therapy for the decision to reimburse the therapy. Furthermore, this multi-design study will allow us to compare the two main methods, without compromising the scientific value of either of the methods to evaluate applicability for future study designs for BPS/IC research.

Trial Registration ClinicalTrials.gov identifier (NCT number): NCT05518864

Keywords: Bladder pain syndrome/ Interstitial Cystitis; GAG-replenishment therapy, Quality of Life, Aggregated N-of-1 trial

Strengths and limitations of this study

+ By combining the classic RCT with an aggregated N-of-1 trial methodology, the study is suitable for group comparison and for within-comparison. For a rare disease with a heterogenous symptom profile, such as BPS/IC, this is beneficial.
+ The study delivers Level 1 evidence according to the Oxford OCEBM Levels of Evidence.[1]
– The Aggregated N-of-1 trial is a less established research design.
– In the cross-over part in the study there are potential carry-over effects, therefore appropriate washout periods have been incorporated in the study protocol.
– An aggregated N-of-1 trial methodology is only possible in chronic disease and non-curing therapies, which is the case for BPS/IC.

INTRODUCTION

Bladder pain syndrome (BPS/IC) is a symptom-based diagnosis, based on exclusion of other identifiable diseases. It has multiple subtypes defined by the International Society for the Study of Bladder Pain Syndrome (ESSIC).[2] The most severely affected subgroup has disease specific inflammatory lesions, called Hunner lesions and is classified as ESSIC subtype 3 (BPS/IC H+). Hunner lesions can be identified and regularly followed up with urethrocystoscopy according to the European EAU guidelines for routine practice.[3] This subtype accounts for approximately 10-20% of all BPS patients and is therefore a rare subtype of an already rare disease.[4-7] Current trends show that the Hunner lesion subtype could be a disease entity on its own.[8] The specific aetiology of BPS/IC is unknown. Pathological characteristics include influx of immune cells in the bladder wall and an increased urothelial permeability because of a damaged urothelial layer and a disruption of protective glycosaminoglycans (GAGs) on the bladder wall lumen.[9, 10] Repair of this barrier by exogenous replenishment of GAGs, has been a key treatment option for BPS/IC for many years.

Investigating (potential) treatments for BPS/IC is difficult. Randomised controlled trials to evaluate GAG therapy have been tried, but many have failed due to heterogeneity of BPS/IC (no subtyping was used) and failure to include sufficient patient numbers for a powered result.[11-13] In 2015, the reimbursement for GAG-therapy was cancelled in the Netherlands due to this lack of level 1 evidence.

Obtaining level 1 evidence is traditionally performed with a traditional double blinded RCT. Government bodies often rely on this methodology to decide whether to reimburse a therapy. Successfully performing a double blinded RCT in a rare disease with heterogenous symptoms is challenging due to large sample sizes needed and often the study is not representative of the real-life situation when patients have subjective symptoms like pain or when patients have mixed symptoms. The N-of-1 trial methodology is based on the concept that the most ideal control for evaluating efficacy is when both treatment and placebo is evaluated in the individual patient. Because of this, N-of-1 trial methodology is limited to chronic non-curable diseases/symptoms and their treatments (treatment must be continued over time to supress the symptom or the disease). Results of individual N-of-1 trials in a group of patients with a similar disease can be combined to obtain level 1 evidence for this

group. The reliability of this efficacy result goes two-ways: the further this group is stratified, the more representative the efficacy results are for this group. The more the group represents the clinical practise target group, the more it can help to identify potential non-responding and responding subgroups.[14] Because treatment and placebo are evaluated in a single patient, a study needs far less patients (half or even less depending on evaluation cycles) for adequate power. So far, no aggregated N-of-1 trial has been directly compared with a traditional RCT.

The goal of this study is to investigate the efficacy of intravesical GAG-therapy with Hyaluronic acid 1.6% + Chondroitin sulfate 2% (Ialuril®, Prefill, IBSA, Goodlife) for bladder pain syndrome (BPS/IC) patients with Hunner lesions using a research design that is in accordance with a Level 1 evidence as defined by the Oxford CEBM evidence grading table.[1]

This study was initiated after discussions between the Dutch Urology Association (NVU) and the Dutch Healthcare Insurance Board (ZIN). It will deliver evidence for the decision whether GAG therapy shall be re-reimbursement within the Netherlands.

METHODS AND ANALYSIS

The GETSBI study is a double-blind multi-design multi-centre randomized placebo-controlled trial to assess the short and long-term efficacy of GAG-therapy with Hyaluronic acid 1.6% + Chondroitin sulfate 2% (Ialuril ® Prefill, IBSA, Goodlife) in patients with BPS/IC H+.

For the short-term, as shown in figure 1, the study protocol is primarily based on a standard RCT, but continues as an aggregated N-of-1 trial.[15, 16] The outcome of the RCT design is the primary design for evaluation, with the aggregated N-of-1 design as a backup in case the inclusion numbers are not met for the RCT design.

For the long-term, the study thereafter continues with an open prospective part evaluating the long-term efficacy of GAG-therapy by 1x/4 weeks Ialuril instillation for 6 months. The total follow-up of the study is 54 weeks.

The study is performed at eight sites during two years of recruitment. Eighty patients need to be included. The main inclusion criteria are symptomatic BPS/ICS patients with Hunner lesions on a cystoscopy in the previous three months with a maximum VAS (visual analogue scale) bladder pain score ≥4 on a scale of 0 to 10 during the last three days. See table 1 for inclusion flowchart. On cystoscopy several parameters are routinely evaluated, e.g., the number of Hunner lesions, estimated % of inflammation of the bladder wall, and an overall assessment of the degree of bladder inflammation (5-point Likert scale). Cystoscopy is not only performed at in the last three months before inclusion and start of the therapy, but also after treatment period one and three.

The primary objective is the maximum VAS bladder pain score in the last 3 days on a scale of 0 to 10. Because of the heterogeneity of symptoms in patients, efficacy of GAG therapy for BPS/IC with Hunner lesions is defined by three possibilities: 1) an improvement of 2 points on the VAS pain score, or 2) an improvement of 2 points on the VAS score on the most dominant symptom that is reported by individual patient, or 3) an improvement of ≥5 on a 7-point Global Response Assessment (GRA) scale.

These are parameters used in literature as primary outcome measures for success of treatment. The improvement of 2 points on the VAS pain score and the most dominant symptom was established by an interview with a patient panel, to consider the heterogeneous symptoms in BPS/IC patient. The GRA scale has been previously used as

primary outcome measure in different RCT's for BPS/IC treatments and gives a patient reported overall assessment of treatment satisfaction.

Secondary study outcomes are improvement on cystoscopy (degree of inflammation and estimate % bladder covered by Hunner lesions), O'Leary Sant Interstitial Cystitis Symptom and Problem Index (OS ICSI / PI), and Patient Reported Outcome Measurement (PROM) . Moreover, specific burden by therapy and start/stop of other BPS treatments will be registered. Cost effectiveness parameters derived from the Medical Consumption Questionnaire (iMCQ) and Productivity Cost Questionnaire (iPCQ). Quality of Life will be assessed by the EQ-5D 5L. All study parameters will be (automatically) filled in the secure electronic database (Castor EDC).

Analysis of covariance (ANCOVA) will be used for the VAS pain score as primary outcome measurement, with baseline as covariate. For the aggregated N-of-1 trial Hierarchical Bayesians modelling is used for statistical analysis. All patients who completed at least one treatment and one placebo period will be included within the aggregated analysis, with inclusion of all available data.

Power calculation

For power calculations, data from Cervigni et al 2017 and Nickel et al 2012 study were used.[12, 13] These studies resemble our study protocol most with regard to the investigational product (Hyaluronic acid + Chondroitin sulfate or Chondroitin sulfate alone), the primary outcome parameters (VAS pain) and RCT design with relative high numbers of inclusion (110 and 98 patients respectively). Both studies included all BPS/IC subtypes. Cervigni used the same HA-CS instillation that is used in this study and showed a treatment effect of approximately -4 on a VAS pain scale (0-10) in comparison with unblinded DMSO instillations. The Nickel study (placebo controlled) showed a placebo effect of approximately -2 on VAS pain scale (0-10). We calculated a SD of 2.62 from the Cervigni study. We used a two-sided test and an alpha of 0.05. Using these estimates for the power calculations (>80%), we would require 58 patients in total to detect a significant difference with placebo. The Nickel and Cervigni studies reported a drop-out ratio between 17 - 25 % for 11 to 24 weeks follow-up (25% would be 15 patients in our study). These studies included all BPS subtypes, we only include the Hunner lesion BPS subtype in our study. This latter subgroup

has more severe symptoms compared to the other subtypes and have therefore a higher risk of drop-out.[17] We also have a longer follow up. Therefore, we increased our inclusion with 22 to a total of 80 patients. In summary, the study will be powered (>80%) for a standard RCT (n=80; consist of 58 + 22 to compensate for potential dropouts). For the aggregated-N-of-1 trial design, >80% power will be achieved at 28 patients, considering the drop-out numbers, the required sample size was calculated at a total of 38 patients.

Recruitment and randomization

The study will be advertised via the Dutch Patient Association for BPS/IC: ICP, by the Dutch Association for Urology (NVU) and participating research centres. The information about the study will be explained and the patient will receive written patient information and will have ample opportunity to ask questions. After this, they can decide to participate and sign the informed consent form. After a patient has been found eligible for inclusion and signed the informed consent, they will be registered in the secure electronic database (Castor EDC software: according to GMC guidelines). Patients will receive a code and key and is then randomized (software generated). After randomization, local investigator can view the randomization outcome in Castor EDC. Local investigator coordinates with the local pharmacy department to prepare investigated product and/or placebo according to the allocated treatment schedule. In case needed the local investigator can perform deblinding in collaboration with the sponsor.

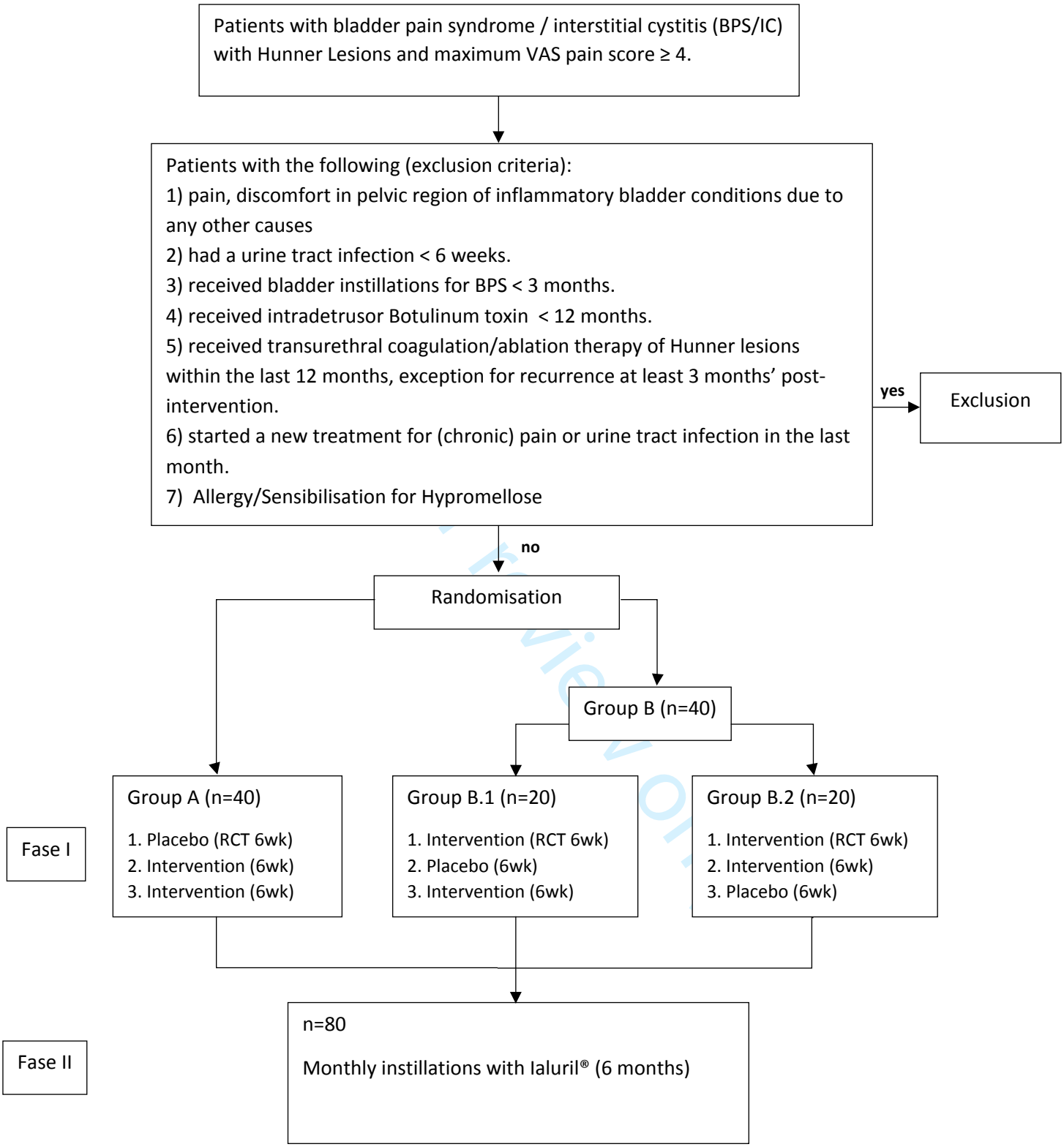
We used the SPIRIT reporting guidelines.[18]

Patient and Public Involvement

For this study a stakeholders workgroup has been established to discuss the study and progress. One of the parties in the stakeholder workgroup is the Dutch Patient Association for BPS/IC: 'Interstitiële Cystitis Patiëntenvereniging' (ICP).

Table 1. Table for inclusion.

Criteria for in-/exclusion and randomization in three parallel study arms.



DISCUSSION

Bladder pain syndrome is a symptom-based diagnosis, based on exclusion of other identifiable diseases. It has multiple subtypes defined by the International Society for the Study of Bladder Pain Syndrome (ESSIC).[2] Subtype 3 is characterized by Hunner lesions. BPS/IC is an orphan disease recognized within the European Research Network for rare diseases (ERN).[19] As a rare disease, it has a low incidence and prevalence. Consequently, this applies even more if only subtype 3 with Hunner lesions is considered. Therefore, the number of patients to be included per study design strongly influence the feasibility of the study to recruit enough patients. Previous RCT's failed to show efficacy of GAG therapy in BPS/IC patients, but these studies did not stratify according to inflammatory and non-inflammatory subtypes (no cystoscopy performed). With inclusion of multiple subtypes, there has been a lot of debate whether these studies had an adequate study design.

Upsides of a standard RCT are the acceptance as the gold standard in clinical research and the use of randomization, double blinding, and placebo-groups for evaluation. It is therefore very suitable to evaluate therapy effects that apply to a group of comparable patients (between-subject comparison). Also, government bodies mostly rely on the traditional RCT for their decision to reimburse a treatment as is the case for this government supported trial.

Relying on evidence from traditional RCT's for reimbursement or guideline advice creates problems for obtaining evidence based and reimbursed healthcare in rare disease. The results of this study could demonstrate and validate the use of aggregated N-of-1 trial methodology to obtain Level 1 evidence with much lower inclusion rates.

The traditional RCT is also often not set-up for being representative for real life clinical practice because of the between subject comparison limitations. To make study patients comparable to each other, strict inclusion/ exclusion criteria are often implemented in the study design. This often leaves out the patients with comorbidities , such as elderly patients who are part of the target population for the investigated treatment.

No direct comparison has been performed between a traditional RCT and an aggregated N-of-1 trial. This trial was therefore set-up to directly compare both study designs without compromising on study outcome measures, study quality and patient burden. It even allows

for a direct comparison with a single-crossover RCT design. The study protocol allowed for: 1) double blinding, 2) equality between participating patients receiving similar amounts of treatment and placebo and 3) the ability to give each participating patient an individual efficacy results at the end of the study.

ETHICS AND DISSEMENATION

The GETSBI study will be conducted according to the principles of the Declaration of Helsinki (seventh version, 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The study will be advertised via the Dutch Patient Association for BPS/IC: ICP, by the Dutch Association for Urology (NVU) and participating research centres. The information about the study will be explained and the patient will receive written patient information and will have ample opportunity to ask questions. After this, they can decide to participate and sign the informed consent form. Benefits for participating in this study are direct reimbursement for the treatment (that is currently not reimbursed) for the duration of the study plus afterwards in the time being the government makes the final decision on reimbursement. Risks are the 6 weeks within the 3.5-month evaluation in which patients get placebo treatment. Considering the duration that patients do get active treatment, this is acceptable in relation to the burden. Therapies are known to be safe with no SAE in previous studies.

Results will be presented at scientific meetings and published in international peer-reviewed journals.

CONCLUSION

Implementing a RCT- with a aggerated N-of-trial design in one study protocol allows not only to determine the efficacy and cost effectiveness of GAG therapy in BPS/IC H+ patients as a rare disease, but also to directly compare the 3 trial methodologies to obtain level 1 evidence (standard RCT, aggregated N-of-1 trial and single crossover RCT design), without compromising the scientific value of either of the methods to evaluate applicability for future trial designs for BPS/IC and other chronic diseases.

FIGURE LEGENDS

Figure 1. Trial Flow Chart

The patients will be randomized in one of the three parallel arms, receiving blinded treatment for three periods, consisting of 1x/week bladder instillation for six weeks. The ratio of intervention to placebo periods is 2:1. In between treatments there are wash-out periods for 4 weeks.

LIST OF ABBREVIATIONS

BPS/IC	Bladder pain syndrome / Interstitial Cystitis
RCT	Randomized Controlled Trial
NVU	Nederlandse Vereniging voor Urologie/ Dutch Urology Association
ESSIC	International Society for the Study of Bladder Pain Syndrome
BPS/IC H+	Bladder pain syndrome/ Interstitial Cystitis with Hunner lesions.
GAGs	Glycosaminoglycans
VAS	visual analogue scale
GRA	Global Response Assessment
OS ICSI/PI	O'Leary Sant Interstitial Cystitis Symptom and Problem Index
PROM	Patient Reported Outcome Measurement
iMCQ	Medical Consumption Questionnaire
iPCQ	Productivity Cost Questionnaire
ANCOVA	Analysis of covariance
ERN	European Research Network for rare diseases

DECLARATIONS

- **Ethics approval and consent to participate**

Title: GAG-therapy Efficacy Trial Solution for Bladder pain syndrome/ Interstitial cystitis
Filenumber CMO : 2020-7265

The medical ethical reviewing committee CMO Regio Arnhem-Nijmegen has reviewed the above mentioned research file on the grounds of section 2, paragraph 2, sub a of the Medical Research Involving Human Subjects Act (WMO).

The committee has approved the research file on April 20, 2021. The decision is based on the documents mentioned in appendix 1 of the original decision written in Dutch.

With kind regards,
On behalf of the CMO Region Arnhem-Nijmegen
Drs. R.B. Keus, vice-chairman

- **Competing interests**: There is an in-kind contribution of Goodlife Pharma BV to this study.
- **Funding**: The trial is funded by the ZonMW 'voorwaardelijke vergoedingen program' and endorsed by the Dutch minister of VWS. There is an in-kind contribution of Goodlife Pharma BV.
- **Authors Contributions**: DJ initiated this study in collaboration with the government. DJ, FM, CG and JH all contributed in the design of the study. CG has written this manuscript. CB, DJ, FM, JH reviewed the manuscript. All authors read and approved the final manuscript.
- **Acknowledgements**: No acknowledgements
- **Data statement**: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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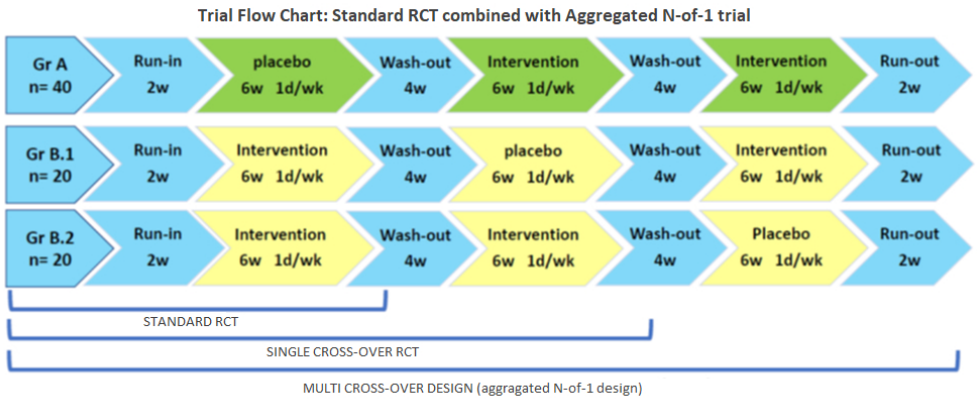

The patients will be randomized in one of the three parallel arms, receiving blinded treatment for three periods, consisting of 1x/week bladder instillation for six weeks. The ratio of intervention to placebo periods is 2:1. In between treatments there are wash-out periods for 4 weeks.

662x292mm (38 x 38 DPI)

Radboudumc

Bijlage 1

Documenten:

A Aanbiedingsbrief d.d. 20 december 2020 (2x)
Bevestiging Eudra CT number d.d. 8 oktober 2020
Aanbiedingsbrief d.d. 20 december 2020 (ontvangen d.d. 26 maart 2021) in reactie op commissievragen d.d. 9 februari 2021
Aanbiedingsbrief d.d. 14 april 2021 in reactie op commissievragen d.d. 13 april 2021
Aanbiedingsbrief d.d. 16 april 2021 in reactie op commissievragen d.d. 15 april 2021

B ABR-formulier, versie 10 d.d. 14 april 2021

C Onderzoeksprotocol, versie 3 d.d. 14 april 2021

D Investigator's Brochure:
- IALURIL
- Methylcellulose Thea (0,5%) = placebo product
- Cystistad
- Gepan Instill
- INSTYLAN
Etiket informatie, ontvangen d.d. 21 december 2020
Apothekershandeling en bereiding, ontvangen d.d. 21 december 2020

E Proefpersoneninformatie incl. toestemmingsformulier, versie 2.0 d.d. 26 februari 2020, ontvangen d.d. 14 april 2021 (geen versiebeheer doorgevoerd bij wijzigingen)
Promotiemateriaal, geen versiebeheer toegepast in het document, ontvangen d.d. 14 april 2021

F Vragenlijsten en meetinstrumenten, ontvangen d.d. 21 december 2020
Patiëntenkaart, ontvangen d.d. 21 december 2020 en d.d. 26 maart 2021

G WMO-proefpersonenverzekering van Radboudumc: van verzekeringsmaatschappij Centramed d.d. januari 2020, datum afgifte RTC CS d.d. 21 december 2020
Bewijs dekking aansprakelijkheid van Radboudumc: van verzekeringsmaatschappij Centramed d.d. januari 2020

H CV onafhankelijk arts L.L. de Wall
CV's lokale onderzoekers compleet

I Lijst deelnemende centra, versie 2
Diverse intentieverklaringen van de deelnemende centra, ontvangen d.d. 21 december 2020
Onderzoeksverklaring van het Radboudumc te Nijmegen, getekend door afdelingshoofd Urologie P.F.A. Mulders d.d. 30 maart 2021 inclusies CV lokale onderzoeker dr. D.A.W. Janssen

J Aanvullende informatie financiële vergoedingen aan onderzoekers en deelnemende centra, versie 2

K Brief minister voor Medische Zorg d.d. 10 oktober 2019
Brief ZonMw voorgenomen besluit subsidieaanvraag d.d. 28 november 2019
Brief minister voor Medische Zorg d.d. 15 november 2019
Draft onderzoekscontract, ongetekend

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2

Protocol version	#3	Date and version identifier	N/A Described in protocol, but not in manuscript.
Funding	#4	Sources and types of financial, material, and other support	13
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	13
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A Described in protocol, but not in manuscript.
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A Described in protocol, but not in manuscript.
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if	N/A Described in protocol, but not in manuscript.

		applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4,5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6,7
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6,7

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6,9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A Described in protocol, but not in manuscript.
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A Described in protocol, but not in manuscript.
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome.	6,7

		Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6,7,9
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7,8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity,	6,7

		if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A Described in protocol, but not in manuscript.
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	7
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	7
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	7
Methods: Monitoring			

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A Described in protocol, but not in manuscript.
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A Described in protocol, but not in manuscript.
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A Described in protocol, but not in manuscript.
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2, 11, 13

Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A Described in protocol, but not in manuscript.
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A Described in protocol, but not in manuscript.
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13

Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A Described in protocol, but not in manuscript.
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A Described in protocol, but not in manuscript.
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A Described in protocol, but not in manuscript.

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A Described in protocol, but not in manuscript.

BMJ Open

A Multicentre double blind RCT, comparing a traditional RCT with an aggregated N-of-1 trial: GAG-therapy Efficacy Trial Solution for Bladder pain syndrome/ Interstitial Cystitis (GETSBI study).

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Abstract

Introduction Obtaining level 1 evidence on efficacy of glycosaminoglycan-therapy (GAG-therapy) is difficult, due to low incidence of bladder pain syndrome (BPS/IC) and heterogeneous symptoms experienced by BPS/IC patients. Currently, because of a lack of high-grade evidence, the recommendation for applying GAG-therapy in most guidelines is 'low grade'. An Aggregated N-of-1 trial is a multi-crossover design that yields similar level 1 evidence as a traditional Randomized Controlled Trial (RCT), while requiring far less patients. The goal of this study is to investigate the efficacy of intravesical GAG-therapy (IALURIL®) for BPS/IC patients with Hunner lesions using a dual RCT and aggregated N-of-1 trial design to obtain Level 1 evidence.

Methods and analysis The GETSBI study is a double-blind multi-design multi-centre randomized placebo-controlled study to assess the short and long-term efficacy of Hyaluronic acid (1.6%) + Chondroitin sulfate (2%) therapy (Ialuril ® Prefill, IBSA, Goodlife) in symptomatic BPS/IC patients with Hunner lesions. It starts as a standard RCT (n=80), but continues as an aggregated N-of-1 trial. There are three parallel arms, receiving blinded treatment for three periods (1x/week for six weeks, ratio placebo to intervention in periods of 2:1). Followed by an open prospective part for the long-term efficacy. The primary study outcome is the maximum bladder pain experienced in the last 3 days measured using the visual analogue pain scale (0-10).

This study is a collaboration with the Dutch government and will deliver evidence for the decision to reimburse the therapy. Furthermore, this multi-design study will allow us to compare the two main methods to evaluate applicability for future study designs for BPS/IC research.

Ethics and dissemination Ethical approval was given by METC Oost-Nederland, file number: 2020-7265, NL-number: NL76290.091.20. Findings from this study will be disseminated via publication, reports and conference presentations.

Trial Registration ClinicalTrials.gov identifier (NCT number): NCT05518864

Keywords: Bladder pain syndrome/ Interstitial Cystitis; GAG-replenishment therapy, Quality of Life, Aggregated N-of-1 trial

Strengths and limitations of this study

- \+ By combining the classic RCT with an aggregated N-of-1 trial methodology, the study is suitable for group comparison and for within-comparison. For a rare disease with a heterogenous symptom profile, such as BPS/IC, this is beneficial.
- \+ The study delivers Level 1 evidence according to the Oxford OCEBM Levels of Evidence.
- – The Aggregated N-of-1 trial is a less established research design.
- – In the cross-over part in the study there are potential carry-over effects, therefore appropriate washout periods have been incorporated in the study protocol.
- – An aggregated N-of-1 trial methodology is only possible in chronic disease and non-curing therapies, which is the case for BPS/IC.

INTRODUCTION

Bladder pain syndrome (BPS/IC) is a symptom-based diagnosis, based on exclusion of other identifiable diseases. It has multiple subtypes defined by the International Society for the Study of Bladder Pain Syndrome (ESSIC) [1]. The most severely affected subgroup has disease specific inflammatory lesions, called Hunner lesions and is classified as ESSIC subtype 3 (BPS/IC HL+). Hunner lesions can be identified and regularly followed up with urethrocystoscopy according to the European EAU guidelines for routine practice [2]. This subtype accounts for approximately 10-20% of all BPS patients and is therefore a rare subtype of an already rare disease [3-6]. Current trends show that the Hunner lesion subtype could be a disease entity on its own [7]. The specific aetiology of BPS/IC is unknown. Pathological characteristics include influx of immune cells in the bladder wall and an increased urothelial permeability because of a damaged urothelial layer and a disruption of protective glycosaminoglycans (GAGs) on the bladder wall lumen [8, 9]. Repair of this barrier by exogenous replenishment of GAGs, has been a key treatment option for BPS/IC for many years.

Investigating (potential) treatments for BPS/IC is difficult. Randomised controlled trials (RCT) to evaluate GAG-therapy have been tried, but many have failed due to heterogeneity of BPS/IC (no subtyping was used) and failure to include sufficient patient numbers for a powered result [10-12]. In 2015, the reimbursement for GAG-therapy was cancelled in the Netherlands due to this lack of level 1 evidence.

Obtaining level 1 evidence is traditionally performed with a traditional double blinded RCT. Government bodies often rely on this methodology to decide whether to reimburse a therapy. Successfully performing a double blinded RCT in a rare disease with heterogenous symptoms is challenging due to large sample sizes needed and often the study is not representative of the real-life situation when patients have subjective symptoms like pain or when patients have mixed symptoms. The N-of-1 trial methodology is based on the concept that the most ideal control for evaluating efficacy is when both treatment and placebo is evaluated in the individual patient. Because of this, N-of-1 trial methodology is limited to chronic non-curable diseases/symptoms and their treatments (treatment must be continued over time to supress the symptom or the disease). Results of individual N-of-1 trials in a group of patients with a similar disease can be combined to obtain level 1 evidence

for this group. The reliability of this efficacy result goes two-ways: the further this group is stratified, the more representative the efficacy results are for this group. The more the group represents the clinical practise target group, the more it can help to identify potential non-responding and responding subgroups [13]. Because treatment and placebo are evaluated in a single patient, a study needs far less patients (half or even less depending on evaluation cycles) for adequate power. So far, no aggregated N-of-1 trial has been directly compared with a traditional RCT.

The goal of this study is to investigate the efficacy of intravesical GAG-therapy with Hyaluronic acid 1.6% + Chondroitin sulfate 2% (Ialuril®, Prefill, IBSA, Goodlife) for BPS/IC HL+ patients using a research design that is in accordance with a Level 1 evidence as defined by the Oxford CEBM evidence grading table [14].

This study was initiated after discussions between the Dutch Urology Association (NVU) and the Dutch Healthcare Insurance Board (ZIN). It will deliver evidence for the decision whether GAG therapy shall be re-reimbursement within the Netherlands.

METHODS AND ANALYSIS

The GETSBI study is a double-blind multi-design multi-centre randomized placebo-controlled trial to assess the short and long-term efficacy of GAG-therapy with Hyaluronic acid 1.6% + Chondroitin sulfate 2% (Ialuril ® Prefill, IBSA, Goodlife) in patients with BPS/IC H+.

For the short-term, as shown in figure 1, the study protocol is primarily based on a standard RCT, but continues as an aggregated N-of-1 trial [15, 16]. The outcome of the RCT design is the primary design for evaluation, with the aggregated N-of-1 design as a backup in case the inclusion numbers are not met for the RCT design.

For the long-term, the study thereafter continues with an open prospective part evaluating the long-term efficacy of GAG-therapy by 1x/4 weeks Ialuril instillation for 6 months. The total follow-up of the study is 54 weeks.

The study is performed at eight sites during two years of recruitment. Eighty patients need to be included. The inclusion criteria are adult (>18 year) symptomatic BPS/IC patients with Hunner lesions on a cystoscopy in the previous three months with a maximum VAS (visual analogue scale) bladder pain score ≥4 on a scale of 0 to 10 during the last three days. The following exclusion criteria are maintained: 1) pain, discomfort in pelvic region of inflammatory bladder conditions due to any other causes based on patients' medical history and interview. There are exceptions for irritable bowel syndrome, hypertonic pelvic floor and urinary tract infections fewer than 3 per year. These are noted by ESSIC as a confusable diseases. 2) had a urinary tract infection < 6 weeks, 3) received bladder instillations for BPS < 3 months, 4) received intra-detrusor botulinum toxin injections < 12 months, 5) received transurethral coagulation/ablation therapy of Hunner Lesions < 12 months, except for patients who have objectified Hunner lesions recurrence on cystoscopy after coagulation/ablation therapy after at least 3 months post-intervention. 6) Started a new treatment for (chronic) pain or urinary tract infections in the last month (after one-month stable use they can be included), 7) unable (also legal) to give informed consent, 8) allergy/sensibilisations for Hypromellose (this will be tested by applying one drop in one eye). See figure 2 for inclusion flowchart.

The primary objective is the maximum VAS bladder pain score in the last 3 days on a scale of 0 to 10.

Secondary outcome measurements are: 1) average VAS bladder pain score in the last 3 days on a scale of 0 to 10, 2) 7-point Global Response Assessment (GRA) scale, 3) VAS dominant symptom burden score (0-10) (for 2 most dominant symptoms), 4) Voiding urgency as single item from the validated OS ICSI/PI questionnaire (5-point Likert Scale), 5) Voiding frequency as single item from the validated OS ICSI/PI questionnaire (5-point Likert Scale), 6) O'Leary Sant Interstitial Cystitis Symptom and Problem Index (OS ICSI / PI), 7) Patient Reported Outcome (PRO) measurement short form. This includes documentation specific burden by therapy and start/stop of other BPS treatment. 8) 2x24h voiding diary, 9) EQ-5D 5L Quality of Life questionnaire, 10) urine sediment for screening bacterial UTI, 11) PRO measurement extended version This includes adverse events (AE) reporting and documentation of start/stop of other BPS treatments. 12). Cost effectiveness parameters derived from the Medical Consumption Questionnaire (iMCQ) and Productivity Cost Questionnaire (iPCQ) and finally 13) Urethrocystoscopically evaluated parameters: number of Hunner Lesions, estimated % of inflammation of bladder wall (VAS scale 0-100%) and overall assessment of degree of bladder inflammation (5-point Likert scale). They are measured at time points: week 0, week 8 and week 28. The cystoscopy parameters are secondary outcome measurements, where the 1) change in estimated percentage of inflammation of the bladder wall (area covered by HL) and 2) change in grade of inflammation will be independently evaluated. Moreover, also the correlations between these two secondary outcomes measurements will be investigated.

Efficacy and statistical analysis

Because of the heterogeneity of symptoms in patients, efficacy of GAG therapy for BPS/IC with Hunner lesions is defined by three possibilities: 1) an improvement of 2 points on the VAS pain score, or 2) an improvement of 2 points on the VAS score on the most dominant symptom that is reported by individual patient, or 3) an improvement of ≥5 on a 7-point GRA-scale. These are parameters used in literature as primary outcome measures for success of treatment. The improvement of 2 points on the VAS pain score and the most dominant symptom was established by an interview with a patient panel, to consider the heterogeneous symptoms in BPS/IC patient. The GRA-scale has been previously used as primary outcome measure in different RCT's for BPS/IC treatments and gives a patient reported overall assessment of treatment satisfaction.

Analysis of covariance (ANCOVA) will be used for the VAS pain score as primary outcome measurement, with baseline as covariate. For the aggregated N-of-1 trial Hierarchical Bayesians modelling is used for statistical analysis. All patients who completed at least one treatment and one placebo period will be included within the aggregated analysis, with inclusion of all available data.

Power calculation

For power calculations, data from Cervigni et al 2017 and Nickel et al 2012 study were used [11, 12]. These studies resemble our study protocol most regarding the investigational product (Hyaluronic acid + Chondroitin sulfate or Chondroitin sulfate alone), the primary outcome parameters (VAS pain) and RCT design with relative high numbers of inclusion (110 and 98 patients respectively). Both studies included all BPS/IC subtypes. Cervigni used the same HA-CS instillation that is used in this study and showed a treatment effect of approximately -4 on a VAS pain scale (0-10) in comparison with unblinded DMSO instillations. The Nickel study (placebo controlled) showed a placebo effect of approximately -2 on VAS pain scale (0-10). We calculated a SD of 2.62 from the Cervigni study. We used a two-sided test and an alpha of 0.05. Using these estimates for the power calculations (>80%), we would require 58 patients in total to detect a significant difference with placebo. The Nickel and Cervigni studies reported a drop-out ratio between 17 - 25 % for 11 to 24 weeks follow-up (25% would be 15 patients in our study). These studies included all BPS subtypes, we only include the Hunner lesion BPS subtype in our study. This latter subgroup has more severe symptoms compared to the other subtypes and have therefore a higher risk of drop-out [17]. We also have a longer follow up. Therefore, we increased our inclusion with 22 to a total of 80 patients. In summary, the study will be powered (>80%) for a standard RCT (n=80; consist of 58 + 22 to compensate for potential dropouts). For the aggregated-N-of-1 trial design, >80% power will be achieved at 28 patients, considering the drop-out numbers, the required sample size was calculated at a total of 38 patients.

Recruitment and randomization

The study will be advertised via the Dutch Patient Association for BPS/IC: ICP, by the Dutch Association for Urology (NVU) and participating research centres. The information about the

study will be explained and the patient will receive written patient information and will have ample opportunity to ask questions. After this, they can decide to participate and sign the informed consent form. After a patient has been found eligible for inclusion and signed the informed consent, they will be registered in the secure electronic database (Castor EDC software: according to GMC guidelines). Patients will receive a code and key and is then randomized (software generated). After randomization, local investigator can view the randomization outcome in Castor EDC. Local investigator coordinates with the local pharmacy department to prepare investigated product and/or placebo according to the allocated treatment schedule. In case needed the local investigator can perform deblinding in collaboration with the sponsor.

We used the SPIRIT reporting guidelines [18].

Patient and Public Involvement

For this study a stakeholder's workgroup has been established to discuss the study and progress. One of the parties in the stakeholder workgroup is the Dutch Patient Association for BPS/IC: 'Interstitiële Cystitis Patiëntenvereniging' (ICP).

CONSIDERATIONS METHODOLOGY

Bladder pain syndrome is a symptom-based diagnosis, based on exclusion of other identifiable diseases. It has multiple subtypes defined by the International Society for the Study of Bladder Pain Syndrome (ESSIC) [1]. Subtype 3 is characterized by Hunner lesions. BPS/IC is an orphan disease recognized within the European Research Network for rare diseases (ERN) [19]. As a rare disease, it has a low incidence and prevalence. Consequently, this applies even more if only subtype 3 with Hunner lesions is considered. Therefore, the number of patients to be included per study design strongly influence the feasibility of the study to recruit enough patients. Previous RCT's failed to show efficacy of GAG therapy in BPS/IC patients, but these studies did not stratify according to inflammatory and non-inflammatory subtypes (no cystoscopy performed). With inclusion of multiple subtypes, there has been a lot of debate whether these studies had an adequate study design. Therefore, in collusion, and as a requirement of the Dutch government for objective measurements, we decided to only include BPS/IC patients with Hunner Lesions to specify

and make the study population more homogenous. Therefore, the addition of the aggregated N-of-1 trial is important as a back-up when inclusions do not meet the power according to the RCT. This study protocol allows for: 1) double blinding, 2) equality between participating patients receiving similar amounts of treatment and placebo and 3) the ability to give each participating patient an individual efficacy results at the end of the study.

The traditional RCT has upsides and downsides. Upsides of a standard RCT are the acceptance as the gold standard in clinical research and the use of randomization, double blinding, and placebo-groups for evaluation. It is therefore very suitable to evaluate therapy effects that apply to a group of comparable patients (between-subject comparison). Also, government bodies mostly rely on the traditional RCT for their decision to reimburse a treatment as is the case for this government supported trial. Downfalls of relying on evidence from traditional RCT's for reimbursement or guideline advice, is firstly seen in obtaining evidence and the reimbursed healthcare in rare disease. Moreover, the traditional RCT is also often not set-up for being representative for real life clinical practice because of the between subject comparison limitations. To make study patients comparable to each other, strict inclusion/ exclusion criteria are often implemented in the study design. This often leaves out the patients with comorbidities , such as elderly patients who are part of the target population for the investigated treatment.

No direct comparison has been performed between a traditional RCT and an aggregated N-of-1 trial. This trial was therefore set-up to directly compare both study designs without compromising on study outcome measures, study quality and patient burden. It even allows for a direct comparison with a single-crossover RCT design. This will be done by actively comparing/evaluating outcome measurements of the study. This study is double blind with appropriate wash-out periods between treatment periods to minimize possible wash-over bias. The study models will be compared on efficacy (significance) level and on correlation level. The primary and secondary outcomes will be evaluated in average changes (with standard deviations) between the models.

ETHICS AND DISSEMENATION

The GETSBI study will be conducted according to the principles of the Declaration of Helsinki (seventh version, 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). Ethical approval was given by METC Oost-Nederland, file number: 2020-7265, NL-number: NL76290.091.20. The study will be advertised via the Dutch Patient Association for BPS/IC: ICP, by the Dutch Association for Urology (NVU) and participating research centres. The information about the study will be explained and the patient will receive written patient information and will have ample opportunity to ask questions. After this, they can decide to participate and sign the informed consent form. Benefits for participating in this study are direct reimbursement for the treatment (that is currently not reimbursed) for the duration of the study plus afterwards in the time being the government makes the final decision on reimbursement. Risks are the 6 weeks within the 3.5-month evaluation in which patients get placebo treatment. Considering the duration that patients do get active treatment, this is acceptable in relation to the burden. Therapies are known to be safe with no SAE in previous studies.

Results will be presented at scientific meetings and published in international peer-reviewed journals.

CONCLUSION

Implementing a RCT- with a aggerated N-of-trial design in one study protocol allows not only to determine the efficacy and cost effectiveness of GAG therapy in BPS/IC HL+ patients as a rare disease, but also to directly compare the 3 trial methodologies to obtain level 1 evidence (standard RCT, aggregated N-of-1 trial and single crossover RCT design), without compromising the scientific value of either of the methods to evaluate applicability for future trial designs for BPS/IC and other chronic diseases.

FIGURE LEGENDS

Figure 1. Trial Flow Chart

The patients will be randomized in one of the three parallel arms, receiving blinded treatment for three periods, consisting of 1x/week bladder instillation for six weeks. The ratio of intervention to placebo periods is 2:1. In between treatments there are wash-out periods for 4 weeks.

Figure 2. Flowchart for inclusion.

Criteria for in-/exclusion and randomization in three parallel study arms.

LIST OF ABBREVIATIONS

BPS/IC	Bladder pain syndrome / Interstitial Cystitis
RCT	Randomized Controlled Trial
NVU	Nederlandse Vereniging voor Urologie/ Dutch Urology Association
ESSIC	International Society for the Study of Bladder Pain Syndrome
BPS/IC H+	Bladder pain syndrome/ Interstitial Cystitis with Hunner lesions.
GAGs	Glycosaminoglycans
VAS	visual analogue scale
GRA	Global Response Assessment
OS ICSI/PI	O'Leary Sant Interstitial Cystitis Symptom and Problem Index
PROM	Patient Reported Outcome Measurement
iMCQ	Medical Consumption Questionnaire
iPCQ	Productivity Cost Questionnaire
ANCOVA	Analysis of covariance
ERN	European Research Network for rare diseases

DECLARATIONS

- **Ethics approval and consent to participate**

Title: GAG-therapy Efficacy Trial Solution for Bladder pain syndrome/ Interstitial cystitis
File number CMO : 2020-7265

The medical ethical reviewing committee CMO Regio Arnhem-Nijmegen has reviewed the above-mentioned research file on the grounds of section 2, paragraph 2, sub a of the Medical Research Involving Human Subjects Act (WMO).

The committee has approved the research file on April 20, 2021. The decision is based on the documents mentioned in appendix 1 of the original decision written in Dutch.

With kind regards,
On behalf of the CMO Region Arnhem-Nijmegen
Drs. R.B. Keus, vice-chairman

- **Competing interests**: There is an in-kind contribution of Goodlife Pharma BV to this study.
- **Funding**: The trial is funded by the ZonMW 'voorwaardelijke vergoedingen program' and endorsed by the Dutch minister of VWS. There is an in-kind contribution of Goodlife Pharma BV. Grant number NA
- **Authors Contributions**: DJ initiated this study in collaboration with the government. DJ, FM, CG and JH all contributed to the design of the study. CG has written this manuscript. CB, DJ, FM, JH reviewed the manuscript. All authors read and approved the final manuscript.
- **Acknowledgements**: No acknowledgements
- **Data statement**: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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15. Punja, S., et al., *N-of-1 trials can be aggregated to generate group mean treatment effects: a systematic review and meta-analysis.* J Clin Epidemiol, 2016. **76**: p. 65-75.
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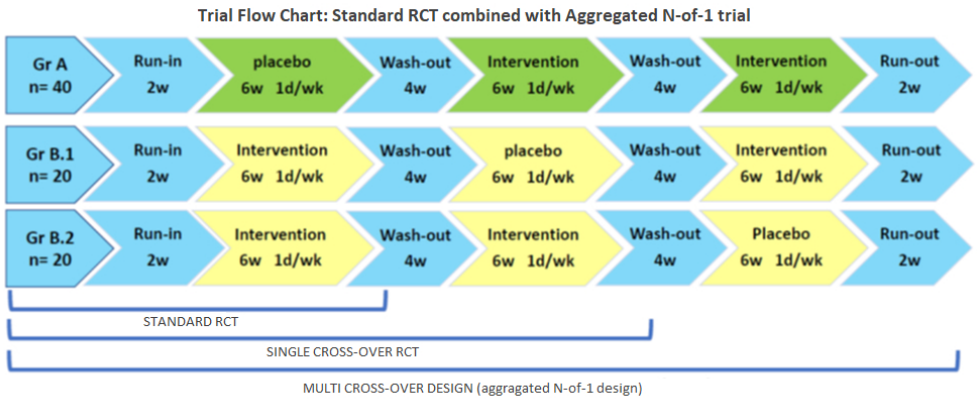
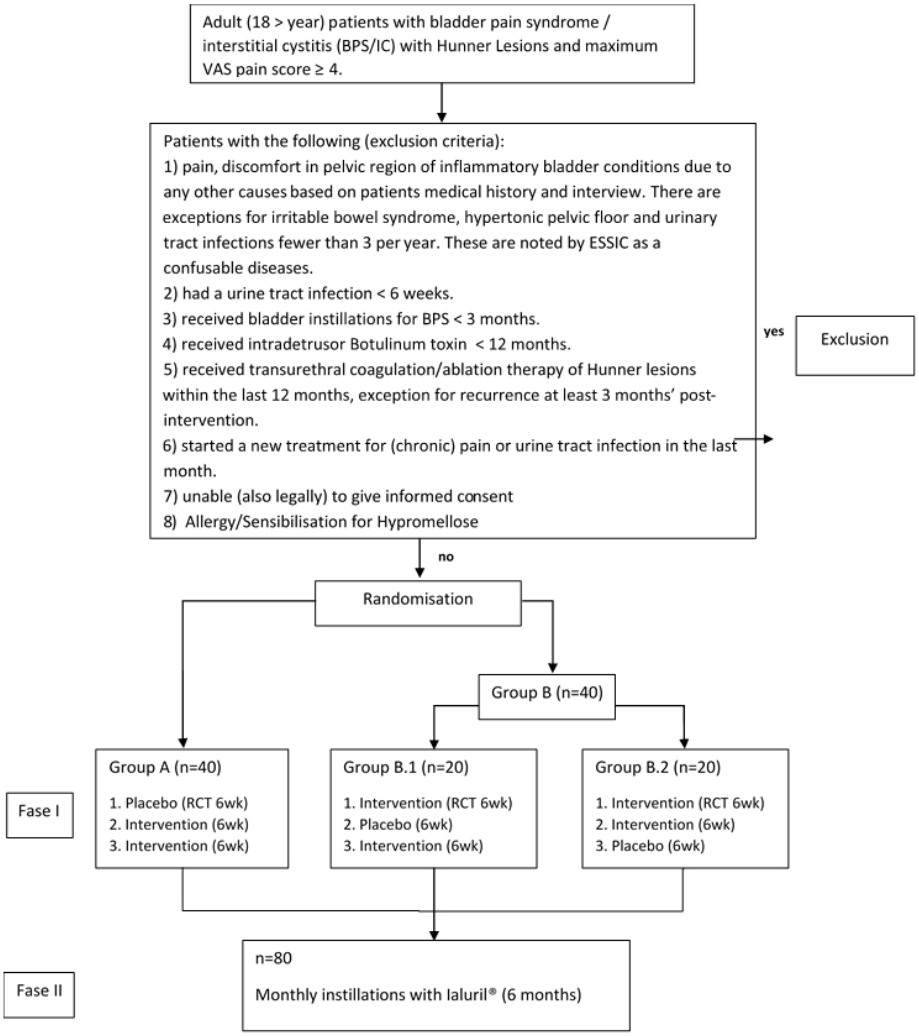
19. eUROGEN, E. *Interstitial Cystitis*. 2016; Available from: https://eurogen-ern.eu/wp-content/uploads/2016/11/IC-Patient-Journey.pdf.



The patients will be randomized in one of the three parallel arms, receiving blinded treatment for three periods, consisting of 1x/week bladder instillation for six weeks. The ratio of intervention to placebo periods is 2:1. In between treatments there are wash-out periods for 4 weeks.

662x292mm (38 x 38 DPI)



Criteria for in-/exclusion and randomization in three parallel study arms.

541x583mm (38 x 38 DPI)

Radboudumc

Bijlage 1

Documenten:

A Aanbiedingsbrief d.d. 20 december 2020 (2x)
Bevestiging Eudra CT number d.d. 8 oktober 2020
Aanbiedingsbrief d.d. 20 december 2020 (ontvangen d.d. 26 maart 2021) in reactie op commissievragen d.d. 9 februari 2021
Aanbiedingsbrief d.d. 14 april 2021 in reactie op commissievragen d.d. 13 april 2021
Aanbiedingsbrief d.d. 16 april 2021 in reactie op commissievragen d.d. 15 april 2021

B ABR-formulier, versie 10 d.d. 14 april 2021

C Onderzoeksprotocol, versie 3 d.d. 14 april 2021

D Investigator's Brochure:
- IALURIL
- Methylcellulose Thea (0,5%) = placebo product
- Cystistad
- Gepan Instill
- INSTYLAN
Etiket informatie, ontvangen d.d. 21 december 2020
Apothekershandeling en bereiding, ontvangen d.d. 21 december 2020

E Proefpersoneninformatie incl. toestemmingsformulier, versie 2.0 d.d. 26 februari 2020, ontvangen d.d. 14 april 2021 (geen versiebeheer doorgevoerd bij wijzigingen)
Promotiemateriaal, geen versiebeheer toegepast in het document, ontvangen d.d. 14 april 2021

F Vragenlijsten en meetinstrumenten, ontvangen d.d. 21 december 2020
Patiëntenkaart, ontvangen d.d. 21 december 2020 en d.d. 26 maart 2021

G WMO-proefpersonenverzekering van Radboudumc: van verzekeringsmaatschappij Centramed d.d. januari 2020, datum afgifte RTC CS d.d. 21 december 2020
Bewijs dekking aansprakelijkheid van Radboudumc: van verzekeringsmaatschappij Centramed d.d. januari 2020

H CV onafhankelijk arts L.L. de Wall
CV's lokale onderzoekers compleet

I Lijst deelnemende centra, versie 2
Diverse intentieverklaringen van de deelnemende centra, ontvangen d.d. 21 december 2020
Onderzoeksverklaring van het Radboudumc te Nijmegen, getekend door afdelingshoofd Urologie P.F.A. Mulders d.d. 30 maart 2021 inclusies CV lokale onderzoeker dr. D.A.W. Janssen

J Aanvullende informatie financiële vergoedingen aan onderzoekers en deelnemende centra, versie 2

K Brief minister voor Medische Zorg d.d. 10 oktober 2019
Brief ZonMw voorgenomen besluit subsidieaanvraag d.d. 28 november 2019
Brief minister voor Medische Zorg d.d. 15 november 2019
Draft onderzoekscontract, ongetekend

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2

Protocol version	#3	Date and version identifier	N/A Described in protocol, but not in manuscript.
Funding	#4	Sources and types of financial, material, and other support	13
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	13
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A Described in protocol, but not in manuscript.
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A Described in protocol, but not in manuscript.
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if	N/A Described in protocol, but not in manuscript.

		applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4,5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6,7
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6,7

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6,9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A Described in protocol, but not in manuscript.
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A Described in protocol, but not in manuscript.
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome.	6,7

		Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6,7,9
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7,8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity,	6,7

		if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A Described in protocol, but not in manuscript.
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	7
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	7
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	7
Methods: Monitoring			

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A Described in protocol, but not in manuscript.
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A Described in protocol, but not in manuscript.
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A Described in protocol, but not in manuscript.
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2, 11, 13

Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A Described in protocol, but not in manuscript.
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A Described in protocol, but not in manuscript.
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13

Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A Described in protocol, but not in manuscript.
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A Described in protocol, but not in manuscript.
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A Described in protocol, but not in manuscript.

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A Described in protocol, but not in manuscript.

BMJ Open

A Study Protocol of a Multicentre double blind RCT, comparing a traditional RCT with an aggregated N-of-1 trial: GAG-therapy Efficacy Trial Solution for Bladder pain syndrome/ Interstitial Cystitis (GETSBI study).

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Abstract

Introduction Obtaining level 1 evidence on efficacy of glycosaminoglycan-therapy (GAG-therapy) is difficult, due to low incidence of bladder pain syndrome (BPS/IC) and heterogeneous symptoms experienced by BPS/IC patients. Currently, because of a lack of high-grade evidence, the recommendation for applying GAG-therapy in most guidelines is 'low grade'. An Aggregated N-of-1 trial is a multi-crossover design that yields similar level 1 evidence as a traditional Randomized Controlled Trial (RCT), while requiring far less patients. The goal of this study is to investigate the efficacy of intravesical GAG-therapy (IALURIL®) for BPS/IC patients with Hunner lesions using a dual RCT and aggregated N-of-1 trial design to obtain Level 1 evidence.

Methods and analysis The GETSBI study is a double-blind multi-design multi-centre randomized placebo-controlled study to assess the short and long-term efficacy of Hyaluronic acid (1.6%) + Chondroitin sulfate (2%) therapy (Ialuril ® Prefill, IBSA, Goodlife) in symptomatic BPS/IC patients with Hunner lesions. It starts as a standard RCT (n=80), but continues as an aggregated N-of-1 trial. There are three parallel arms, receiving blinded treatment for three periods (1x/week for six weeks, ratio placebo to intervention in periods of 2:1). Followed by an open prospective part for the long-term efficacy. The primary study outcome is the maximum bladder pain experienced in the last 3 days measured using the visual analogue pain scale (0-10).

This study is a collaboration with the Dutch government and will deliver evidence for the decision to reimburse the therapy. Furthermore, this multi-design study will allow us to compare the two main methods to evaluate applicability for future study designs for BPS/IC research.

Ethics and dissemination Ethical approval was given by METC Oost-Nederland, file number: 2020-7265, NL-number: NL76290.091.20. Findings from this study will be disseminated via publication, reports and conference presentations.

Trial Registration ClinicalTrials.gov identifier (NCT number): NCT05518864

Keywords: Bladder pain syndrome/ Interstitial Cystitis; GAG-replenishment therapy, Quality of Life, Aggregated N-of-1 trial

Strengths and limitations of this study

+ By combining the classic RCT with an aggregated N-of-1 trial methodology, the study is suitable for group comparison and for within-comparison. For a rare disease with a heterogenous symptom profile, such as BPS/IC, this is beneficial.
+ The study delivers Level 1 evidence according to the Oxford OCEBM Levels of Evidence.
– The Aggregated N-of-1 trial is a less established research design.
– In the cross-over part in the study there are potential carry-over effects, therefore appropriate washout periods have been incorporated in the study protocol.
– An aggregated N-of-1 trial methodology is only possible in chronic disease and non-curing therapies, which is the case for BPS/IC.

INTRODUCTION

Bladder pain syndrome (BPS/IC) is a symptom-based diagnosis, based on exclusion of other identifiable diseases. It has multiple subtypes defined by the International Society for the Study of Bladder Pain Syndrome (ESSIC) [1]. The most severely affected subgroup has disease specific inflammatory lesions, called Hunner lesions and is classified as ESSIC subtype 3 (BPS/IC HL+). Hunner lesions can be identified and regularly followed up with urethrocystoscopy according to the European EAU guidelines for routine practice [2]. This subtype accounts for approximately 10-20% of all BPS patients and is therefore a rare subtype of an already rare disease [3-6]. Current trends show that the Hunner lesion subtype could be a disease entity on its own [7]. The specific aetiology of BPS/IC is unknown. Pathological characteristics include influx of immune cells in the bladder wall and an increased urothelial permeability because of a damaged urothelial layer and a disruption of protective glycosaminoglycans (GAGs) on the bladder wall lumen [8, 9]. Repair of this barrier by exogenous replenishment of GAGs, has been a key treatment option for BPS/IC for many years.

Investigating (potential) treatments for BPS/IC is difficult. Randomised controlled trials (RCT) to evaluate GAG-therapy have been tried, but many have failed due to heterogeneity of BPS/IC (no subtyping was used) and failure to include sufficient patient numbers for a powered result [10-12]. In 2015, the reimbursement for GAG-therapy was cancelled in the Netherlands due to this lack of level 1 evidence.

Obtaining level 1 evidence is traditionally performed with a traditional double blinded RCT. Government bodies often rely on this methodology to decide whether to reimburse a therapy. Successfully performing a double blinded RCT in a rare disease with heterogenous symptoms is challenging due to large sample sizes needed and often the study is not representative of the real-life situation when patients have subjective symptoms like pain or when patients have mixed symptoms. The N-of-1 trial methodology is based on the concept that the most ideal control for evaluating efficacy is when both treatment and placebo is evaluated in the individual patient. Because of this, N-of-1 trial methodology is limited to chronic non-curable diseases/symptoms and their treatments (treatment must be continued over time to supress the symptom or the disease). Results of individual N-of-1 trials in a group of patients with a similar disease can be combined to obtain level 1 evidence

for this group. The reliability of this efficacy result goes two-ways: the further this group is stratified, the more representative the efficacy results are for this group. The more the group represents the clinical practise target group, the more it can help to identify potential non-responding and responding subgroups [13]. Because treatment and placebo are evaluated in a single patient, a study needs far less patients (half or even less depending on evaluation cycles) for adequate power. So far, no aggregated N-of-1 trial has been directly compared with a traditional RCT.

The goal of this study is to investigate the efficacy of intravesical GAG-therapy with Hyaluronic acid 1.6% + Chondroitin sulfate 2% (Ialuril®, Prefill, IBSA, Goodlife) for BPS/IC HL+ patients using a research design that is in accordance with a Level 1 evidence as defined by the Oxford CEBM evidence grading table [14].

This study was initiated after discussions between the Dutch Urology Association (NVU) and the Dutch Healthcare Insurance Board (ZIN). It will deliver evidence for the decision whether GAG therapy shall be re-reimbursement within the Netherlands.

METHODS AND ANALYSIS

The GETSBI study is a double-blind multi-design multi-centre randomized placebo-controlled trial to assess the short and long-term efficacy of GAG-therapy with Hyaluronic acid 1.6% + Chondroitin sulfate 2% (Ialuril ® Prefill, IBSA, Goodlife) in patients with BPS/IC H+.

For the short-term, as shown in figure 1, the study protocol is primarily based on a standard RCT, but continues as an aggregated N-of-1 trial [15, 16]. The outcome of the RCT design is the primary design for evaluation, with the aggregated N-of-1 design as a backup in case the inclusion numbers are not met for the RCT design.

For the long-term, the study thereafter continues with an open prospective part evaluating the long-term efficacy of GAG-therapy by 1x/4 weeks Ialuril instillation for 6 months. The total follow-up of the study is 54 weeks.

The study is performed at eight sites during two years of recruitment. Eighty patients need to be included. The inclusion criteria are adult (>18 year) symptomatic BPS/IC patients with Hunner lesions on a cystoscopy in the previous three months with a maximum VAS (visual analogue scale) bladder pain score ≥4 on a scale of 0 to 10 during the last three days. The following exclusion criteria are maintained: 1) pain, discomfort in pelvic region of inflammatory bladder conditions due to any other causes based on patients' medical history and interview (i.e. bladder pathologies and instillations with irritative agents, such as intravesical chemotherapy). There are exceptions for irritable bowel syndrome, hypertonic pelvic floor and urinary tract infections fewer than 3 per year. These are noted by ESSIC as a confusable diseases. 2) had a urinary tract infection < 6 weeks, 3) received bladder instillations for BPS < 3 months, 4) received intra-detrusor botulinum toxin injections < 12 months, 5) received transurethral coagulation/ablation therapy of Hunner Lesions < 12 months, except for patients who have objectified Hunner lesions recurrence on cystoscopy after coagulation/ablation therapy after at least 3 months post-intervention. 6) Started a new treatment for (chronic) pain or urinary tract infections in the last month (after one-month stable use they can be included), 7) unable (also legal) to give informed consent, 8) allergy/sensibilisations for Hypromellose (this will be tested by applying one drop in one eye). See figure 2 for inclusion flowchart.

The primary objective is the maximum VAS bladder pain score in the last 3 days on a scale of 0 to 10.

Secondary outcome measurements are: 1) average VAS bladder pain score in the last 3 days on a scale of 0 to 10, 2) 7-point Global Response Assessment (GRA) scale, 3) VAS dominant symptom burden score (0-10) (for 2 most dominant symptoms), 4) Voiding urgency as single item from the OS ICSI/PI questionnaire (5-point Likert Scale), 5) Voiding frequency as single item from the OS ICSI/PI questionnaire (5-point Likert Scale), 6) O'Leary Sant Interstitial Cystitis Symptom and Problem Index (OS ICSI / PI), 7) Patient Reported Outcome (PRO) measurement short form. This includes documentation specific burden by therapy and start/stop of other BPS treatment. 8) 2x24h voiding diary, 9) EQ-5D 5L Quality of Life questionnaire, 10) urine sediment for screening bacterial UTI, 11) PRO measurement extended version This includes adverse events (AE) reporting and documentation of start/stop of other BPS treatments. 12). Cost effectiveness parameters derived from the Medical Consumption Questionnaire (iMCQ) and Productivity Cost Questionnaire (iPCQ) and finally 13) Urethrocystoscopically evaluated parameters: number of Hunner Lesions, estimated % of inflammation of bladder wall (VAS scale 0-100%) and overall assessment of degree of bladder inflammation (5-point Likert scale). They are measured at time points: week 0, week 8 and week 28. The cystoscopy parameters are secondary outcome measurements, where the 1) change in estimated percentage of inflammation of the bladder wall (area covered by HL) and 2) change in grade of inflammation will be independently evaluated. Moreover, also the correlations between these two secondary outcomes measurements will be investigated. All questionnaires are in Dutch.

Efficacy and statistical analysis

Because of the heterogeneity of symptoms in patients, efficacy of GAG therapy for BPS/IC with Hunner lesions is defined by three possibilities: 1) an improvement of 2 points on the VAS pain score, or 2) an improvement of 2 points on the VAS score on the most dominant symptom that is reported by individual patient, or 3) an improvement of ≥5 on a 7-point GRA-scale. These are parameters used in literature as primary outcome measures for success of treatment. The improvement of 2 points on the VAS pain score and the most dominant symptom was established by an interview with a patient panel, to consider the heterogeneous symptoms in BPS/IC patient. The GRA-scale has been previously used as

primary outcome measure in different RCT's for BPS/IC treatments and gives a patient reported overall assessment of treatment satisfaction.

Analysis of covariance (ANCOVA) will be used for the VAS pain score as primary outcome measurement, with baseline as covariate. For the aggregated N-of-1 trial Hierarchical Bayesians modelling is used for statistical analysis. All patients who completed at least one treatment and one placebo period will be included within the aggregated analysis, with inclusion of all available data.

Power calculation

For power calculations, data from Cervigni et al 2017 and Nickel et al 2012 study were used [11, 12]. These studies resemble our study protocol most regarding the investigational product (Hyaluronic acid + Chondroitin sulfate or Chondroitin sulfate alone), the primary outcome parameters (VAS pain) and RCT design with relative high numbers of inclusion (110 and 98 patients respectively). Both studies included all BPS/IC subtypes. Cervigni used the same HA-CS instillation that is used in this study and showed a treatment effect of approximately -4 on a VAS pain scale (0-10) in comparison with unblinded DMSO instillations. The Nickel study (placebo controlled) showed a placebo effect of approximately -2 on VAS pain scale (0-10). We calculated a SD of 2.62 from the Cervigni study. We used a two-sided test and an alpha of 0.05. Using these estimates for the power calculations (>80%), we would require 58 patients in total to detect a significant difference with placebo. The Nickel and Cervigni studies reported a drop-out ratio between 17 - 25 % for 11 to 24 weeks follow-up (25% would be 15 patients in our study). These studies included all BPS subtypes, we only include the Hunner lesion BPS subtype in our study. This latter subgroup has more severe symptoms compared to the other subtypes and have therefore a higher risk of drop-out [17]. We also have a longer follow up. Therefore, we increased our inclusion with 22 to a total of 80 patients. In summary, the study will be powered (>80%) for a standard RCT (n=80; consist of 58 + 22 to compensate for potential dropouts). For the aggregated-N-of-1 trial design, >80% power will be achieved at 28 patients, considering the drop-out numbers, the required sample size was calculated at a total of 38 patients.

Recruitment and randomization

The study will be advertised via the Dutch Patient Association for BPS/IC: ICP, by the Dutch Association for Urology (NVU) and participating research centres. The information about the study will be explained and the patient will receive written patient information and will have ample opportunity to ask questions. After this, they can decide to participate and sign the informed consent form. After a patient has been found eligible for inclusion and signed the informed consent, they will be registered in the secure electronic database (Castor EDC software: according to GMC guidelines). Patients will receive a code and key and is then randomized (software generated). After randomization, local investigator can view the randomization outcome in Castor EDC. Local investigator coordinates with the local pharmacy department to prepare investigated product and/or placebo according to the allocated treatment schedule. In case needed the local investigator can perform deblinding in collaboration with the sponsor.

We used the SPIRIT reporting guidelines [18].

Patient and Public Involvement

For this study a stakeholder's workgroup has been established to discuss the study and progress. One of the parties in the stakeholder workgroup is the Dutch Patient Association for BPS/IC: 'Interstitiële Cystitis Patiëntenvereniging' (ICP).

CONSIDERATIONS METHODOLOGY

Bladder pain syndrome is a symptom-based diagnosis, based on exclusion of other identifiable diseases. It has multiple subtypes defined by the International Society for the Study of Bladder Pain Syndrome (ESSIC) [1]. Subtype 3 is characterized by Hunner lesions. BPS/IC is an orphan disease recognized within the European Research Network for rare diseases (ERN) [19]. As a rare disease, it has a low incidence and prevalence. Consequently, this applies even more if only subtype 3 with Hunner lesions is considered. Therefore, the number of patients to be included per study design strongly influence the feasibility of the study to recruit enough patients. Previous RCT's failed to show efficacy of GAG therapy in BPS/IC patients, but these studies did not stratify according to inflammatory and non-inflammatory subtypes (no cystoscopy performed). With inclusion of multiple subtypes, there has been a lot of debate whether these studies had an adequate study design.

Therefore, in collusion, and as a requirement of the Dutch government for objective measurements, we decided to only include BPS/IC patients with Hunner Lesions to specify and make the study population more homogenous. Therefore, the addition of the aggregated N-of-1 trial is important as a back-up when inclusions do not meet the power according to the RCT. This study protocol allows for: 1) double blinding, 2) equality between participating patients receiving similar amounts of treatment and placebo and 3) the ability to give each participating patient an individual efficacy results at the end of the study.

The traditional RCT has upsides and downsides. Upsides of a standard RCT are the acceptance as the gold standard in clinical research and the use of randomization, double blinding, and placebo-groups for evaluation. It is therefore very suitable to evaluate therapy effects that apply to a group of comparable patients (between-subject comparison). Also, government bodies mostly rely on the traditional RCT for their decision to reimburse a treatment as is the case for this government supported trial. Downfalls of relying on evidence from traditional RCT's for reimbursement or guideline advice, is firstly seen in obtaining evidence and the reimbursed healthcare in rare disease. Moreover, the traditional RCT is also often not set-up for being representative for real life clinical practice because of the between subject comparison limitations. To make study patients comparable to each other, strict inclusion/ exclusion criteria are often implemented in the study design. This often leaves out the patients with comorbidities , such as elderly patients who are part of the target population for the investigated treatment.

No direct comparison has been performed between a traditional RCT and an aggregated N-of-1 trial. This trial was therefore set-up to directly compare both study designs without compromising on study outcome measures, study quality and patient burden. It even allows for a direct comparison with a single-crossover RCT design. This will be done by actively comparing/evaluating outcome measurements of the study. This study is double blind with appropriate wash-out periods between treatment periods to minimize possible wash-over bias. The study models will be compared on efficacy (significance) level and on correlation level. The primary and secondary outcomes will be evaluated in average changes (with standard deviations) between the models.

ETHICS AND DISSEMENATION

The GETSBI study will be conducted according to the principles of the Declaration of Helsinki (seventh version, 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO). Ethical approval was given by METC Oost-Nederland, file number: 2020-7265, NL-number: NL76290.091.20. The study will be advertised via the Dutch Patient Association for BPS/IC: ICP, by the Dutch Association for Urology (NVU) and participating research centres. The information about the study will be explained and the patient will receive written patient information and will have ample opportunity to ask questions. After this, they can decide to participate and sign the informed consent form. Benefits for participating in this study are direct reimbursement for the treatment (that is currently not reimbursed) for the duration of the study plus afterwards in the time being the government makes the final decision on reimbursement. Risks are the 6 weeks within the 3.5-month evaluation in which patients get placebo treatment. Considering the duration that patients do get active treatment, this is acceptable in relation to the burden. Therapies are known to be safe with no SAE in previous studies.

Results will be presented at scientific meetings and published in international peer-reviewed journals.

Implementing a RCT- with a aggerated N-of-trial design in one study protocol allows not only to determine the efficacy and cost effectiveness of GAG therapy in BPS/IC HL+ patients as a rare disease, but also to directly compare the 3 trial methodologies to obtain level 1 evidence (standard RCT, aggregated N-of-1 trial and single crossover RCT design), without compromising the scientific value of either of the methods to evaluate applicability for future trial designs for BPS/IC and other chronic diseases.

FIGURE LEGENDS

Figure 1. Trial Flow Chart

The patients will be randomized in one of the three parallel arms, receiving blinded treatment for three periods, consisting of 1x/week bladder instillation for six weeks. The ratio of intervention to placebo periods is 2:1. In between treatments there are wash-out periods for 4 weeks.

Figure 2. Flowchart for inclusion.

Criteria for in-/exclusion and randomization in three parallel study arms.

LIST OF ABBREVIATIONS

BPS/IC	Bladder pain syndrome / Interstitial Cystitis
RCT	Randomized Controlled Trial
NVU	Nederlandse Vereniging voor Urologie/ Dutch Urology Association
ESSIC	International Society for the Study of Bladder Pain Syndrome
BPS/IC H+	Bladder pain syndrome/ Interstitial Cystitis with Hunner lesions.
GAGs	Glycosaminoglycans
VAS	visual analogue scale
GRA	Global Response Assessment
OS ICSI/PI	O'Leary Sant Interstitial Cystitis Symptom and Problem Index
PROM	Patient Reported Outcome Measurement
iMCQ	Medical Consumption Questionnaire
iPCQ	Productivity Cost Questionnaire
ANCOVA	Analysis of covariance
ERN	European Research Network for rare diseases

DECLARATIONS

- **Ethics approval and consent to participate**

Title: GAG-therapy Efficacy Trial Solution for Bladder pain syndrome/ Interstitial cystitis
File number CMO : 2020-7265

The medical ethical reviewing committee CMO Regio Arnhem-Nijmegen has reviewed the above-mentioned research file on the grounds of section 2, paragraph 2, sub a of the Medical Research Involving Human Subjects Act (WMO).

The committee has approved the research file on April 20, 2021.

With kind regards,
On behalf of the CMO Region Arnhem-Nijmegen
Drs. R.B. Keus, vice-chairman

- **Competing interests**: There is an in-kind contribution of Goodlife Pharma BV to this study.
- **Funding**: The trial is funded by the ZonMW 'voorwaardelijke vergoedingen program' and endorsed by the Dutch minister of VWS. There is an in-kind contribution of Goodlife Pharma BV. Grant number NA
- **Authors Contributions**: DJ initiated this study in collaboration with the government. DJ, FM, CG and JH all contributed to the design of the study. CG has written this manuscript. CB, DJ, FM, JH reviewed the manuscript. All authors read and approved the final manuscript.
- **Acknowledgements**: No acknowledgements
- **Data statement**: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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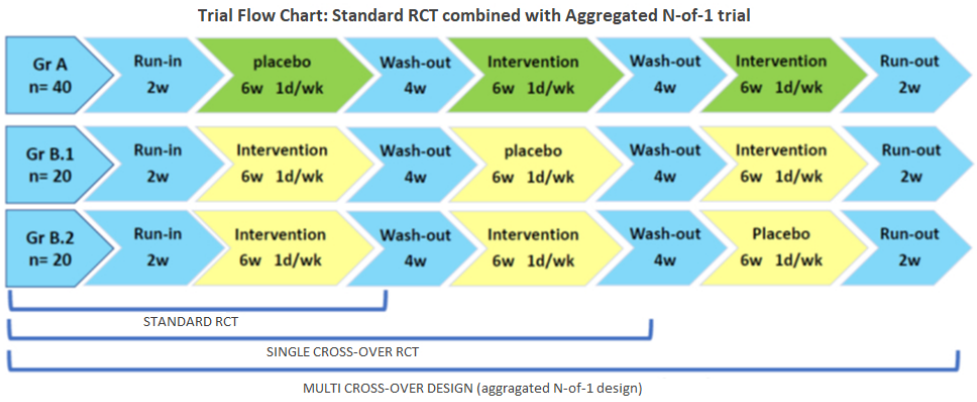

The patients will be randomized in one of the three parallel arms, receiving blinded treatment for three periods, consisting of 1x/week bladder instillation for six weeks. The ratio of intervention to placebo periods is 2:1. In between treatments there are wash-out periods for 4 weeks.

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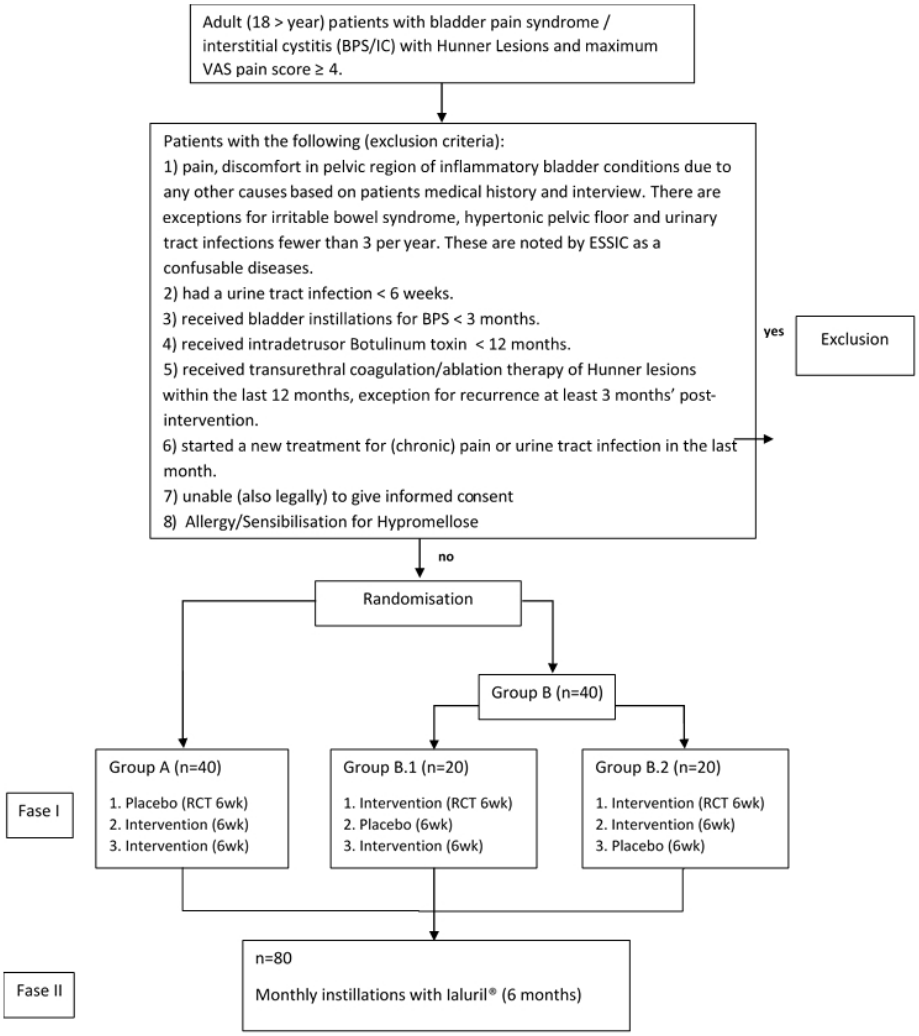

Criteria for in-/exclusion and randomization in three parallel study arms.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2

Protocol version	#3	Date and version identifier	N/A Described in protocol, but not in manuscript.
Funding	#4	Sources and types of financial, material, and other support	13
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	13
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	N/A Described in protocol, but not in manuscript.
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A Described in protocol, but not in manuscript.
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if	N/A Described in protocol, but not in manuscript.

		applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4,5
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6,7
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	6,7

Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	6,9
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A Described in protocol, but not in manuscript.
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A Described in protocol, but not in manuscript.
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome.	6,7

		Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6,7,9
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7,8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8

Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity,	6,7

		if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A Described in protocol, but not in manuscript.
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	7
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	7
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	7
Methods: Monitoring			

Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A Described in protocol, but not in manuscript.
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A Described in protocol, but not in manuscript.
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A Described in protocol, but not in manuscript.
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	2, 11, 13

Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A Described in protocol, but not in manuscript.
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	8
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A Described in protocol, but not in manuscript.
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	13

Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A Described in protocol, but not in manuscript.
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A Described in protocol, but not in manuscript.
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A Described in protocol, but not in manuscript.

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A Described in protocol, but not in manuscript.